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TITLE OF THE INVENTION (280 characters max)

METHOD AND DEVICE FOR DIRECT QUANTITATIVE DETERMINATION OF PESTICIDE SEED LOADING ON INDIVIDUAL SEEDS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

- ☒ Specification (Including Any Claims and Abstract) - 20 pages
- ☐ Drawings - sheets
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Date: October 29, 2003

METHOD AND DEVICE FOR DIRECT QUANTITATIVE DETERMINATION OF PESTICIDE SEED LOADING ON INDIVIDUAL SEEDS

Field Of The Invention

[0001] The invention relates generally to a method and device for pesticide analysis on individual seeds. More particularly to methods and devices for determining quantities of multiple pesticides extracted from seeds of crop plants.

Background Of The Invention

[0002] A reliable determination of pesticide quantities in agricultural and environmental practices is very important. In the agricultural area, pesticides are employed in a variety of ways including coating seeds to protect them against many pests, including being attacked by insects or by soil borne diseases. It is particularly important to control the quantity of pesticides coated on an individual seed because too little pesticide will result in incomplete protection of the seed and emerging plant while too much pesticide may have negative effects on the seed and its germination.

[0003] The current art of quantitative pesticide determination on seeds includes the chromatographic or fluorescence analysis of samples with large numbers of seeds (several grams in weight) or extraction of the dye or pigment from the surface of an individual seed followed by fluorescence analysis. Analysis of larger quantities of seeds shows the average pesticide content of the seeds, however it does not allow the determination of how well each seed is protected on a single seed basis. The present method of quantitation of the dye from a single seed is valid only if the dye content is directly correlated to the pesticide content. This correlation is not always valid, especially in seeds treated with multiple active ingredients having different physico-chemical properties (melting point, water solubility, particle size,...). Also, application of this method is obviously limited to seed treatments which include a fluorescent ingredient that can be extracted from the seeds.

[0004] Current practice in chromatographic analysis of pesticides from coatings of a large sample of seeds could not be successfully translated to multiple A.I. analysis of single seeds due to several key issues.

[0005] Classical methods of A.I. extraction utilized for gram quantities of seed gave incomplete or erratic results at the trace levels required for single seeds.

[0006] The typical manual solvent addition technique used for extracting the seeds was not practical considering the large number of analyses required for a reliable seed-to-seed distribution determination (typically 60 seeds or more).

[0007] Difficulty is encountered when filtering the extract using a syringe equipped with a syringe filter due to the low volume of extract available and the amount retained on the filter.

[0008] Very dilute extracts of the active ingredients from a single seed provided a significantly suboptimal matrix for reproducible chromatographic analysis.

[0009] The limitations of the present art were overcome through a number of experiments which ultimately provided successful results with single seed analysis of commercially important seed treatment active ingredients from crop seeds such as corn, cotton, wheat, soybean and canola.

Summary Of The Invention

[0010] According to the present invention, methods and devices are provided for the quantitative analysis of trace levels of multiple pesticides from a single seed. Direct quantitative determination of multiple active ingredients on a single seed has proved to be a significant challenge due to the extremely low levels of the active ingredients on each individual seed and the difficulty to reproducibly extract these trace quantities. For example, it is not unusual to have loadings as low as 1 µg of each active ingredient on commercially treated seed. Surprisingly, it has now been found that the methods of high-pressure liquid chromatography or gas chromatography can be reproducibly applied to analysis of multiple pesticides from a single

seed by utilization of new, specifically optimized seed preparation, extraction, filtration and detection techniques.

[0011] The method for determining the single seed loading distribution (Gaussian and non-Gaussian) of a pesticide (applicable to multiple pesticides) on a batch of pesticidally treated seeds comprises the steps of:

1) Selecting a subset of seeds from said batch sufficient to determing said distribution;

2) Maintaining a seed from said subset in contact with an extracting fluid in a sufficient volume to comprise a test sample and for a sufficient time to substantially non-selectively extract said pesticide from said seed;

3) Filtering the test sample containing the pesticide to substantially remove undesired substances extracted from the seed while maintaining a sufficient volume for chromatographic analysis

4) Separating the pesticide from other substances in the filtered test sample by chromatography;

5) Illuminating the pesticide with UV light {other detectors e.g., a conductivity detector, RI (Refractive index), ELS (evaporative light scattering) for HPLC for example or FID (flame ionisation detector), ECD (electron capture detector) or TCD (thernal conductivity detector) for GC for example can be used);

6) Detecting the signal produced by the pesticide;

7) Relating the amount of detector signal to a quantity of pesticide;

8) Repeating Steps 2-7 sequentially, or concurrently, for each seed in said subset;

9) Determining the single seed loading distribution (both Gaussian and non-Gaussian) for said batch based on the pesticide quantity determined for each seed in the subset; and

10) Repeat Steps 4-9 to determine quantity and seed loading distribution of each additional pesticide in the test sample.

[0012] Based on the above-described techniques a novel method for the determination of the quantity of pesticides from a single seed has been found. The method comprises the steps of selecting a subset of seeds, extracting the active ingredients from the seed using a specific

solvent system selected based on the active ingredient and seed being extracted, followed by sonication or mechanical shaking, filtration, separation by HPLC or GC, and detection. The method is applicable to determination of a broad range of pesticides when loaded on seeds of key agronomic crops such as corn, cotton, wheat, soybean and canola. Suitable crop seeds to analyzed in accordance with the invention include conventional as well as genetically enhanced or engineered varieties such as, for example, insect resistant (e.g., Bt.) as well as herbicide and disease resistant varieties.

Detailed Description Of The Invention

[0013] According to the present invention, methods and devices are provided for the quantitative analysis of trace levels of multiple pesticides from a single seed. It has now been found that the methods of high-pressure liquid chromatography or gas chromatography can be reproducibly applied to analysis of multiple pesticides from a single seed by utilization of new, specifically optimized seed preparation, extraction, filtration and detection techniques. The method is workable for pigment-free compositions as well as those containing a pigment.

[0014] Key steps made in improvement of the present art are outlined below:

Solvent Choice

[0015] Picking a solvent or a combination of solvents that will reproducibly extract the active ingredients from a single seed is a key step. This combination can be different for the different seeds and active ingredients being tested and is not purely based on the solubility of the active ingredients. For example, thiamethoxam (CGA-293343) needs to be exposed to water in a prewetting or soaking step to be effectively removed from corn seed. After this wetting step the addition of acetonitrile is used to complete the extraction. When extracting thiamethoxam from cotton seeds a mixture of 50:50 water:acetonitrile can be used in one step with no soaking of the seed. The corn extraction scheme was successfully used with no further testing to remove fludioxinil (CGA-173506), mefenoxam (CGA-329351) imidacloprid and metalaxyl (CGA-48988). However, the addition of acetone was required for extraction of captan from corn seeds, due to its low water solubility.

Automation Device

[0016] An automatic solvent addition device that can reproducibly add low volumes of solvent to a large number of samples was required because of the large volume of seeds being analyzed (60 for each analysis). The repetitive use of a manual pipette is not feasible due to excessive operator exposure to the solvent and operator errors that can be introduced in such a repetitive and tedious operation. A Hamilton Autodiluter outfitted with a 5 ml syringe was successfully utilized for this purpose, but other devices may also be workable.

Filtration Technique

[0017] This solution needs to be filtered after extraction but before analysis by a chromatographic technique. Filtering a small volume (e.g., one milliliter) of sample solution while maintaining a sufficient sample volume for chromatographic analysis proved to be difficult. The 13 mm 0.45 micron Acrodisc syringe filters typically used for this purpose retain between 0.5ml and 1.0 ml, leaving an inadequate amount to fill the autosampler vial. The solution found involved using a Whatmann Uniprep vial which is an autosampler vial with a built-in filter.

Signal Optimization

[0018] Optimizing the signal to allow for detection of the active ingredients, especially when multiple active ingredients were being determined from one seed, proved to be a major hurdle. Concentrations from extraction of a single seed coated with 10 ppm of pesticide using one milliliter of solvent would lead to a concentration of about 0.0002 mg/ml. A typical injection volume of 10 microliters into the chromatograph would require detection of amounts as small as 0.2 nanograms of pesticide. Normally when faced with trace level analysis, a chemist can increase the sample size. Since the method is by definition analysis of one seed this was not possible.

[0019] The following steps were taken to improve the sensitivity of the method:

[0020] Most pesticide components can be detected at either 265 nm or 230 nm wavelength. Use of a dual channel detector allows simultaneous detection at both wavelengths thereby optimizing the detection signals.

[0021] Increased injection volume to 15 ul to increase amount of pesticide detected.

[0022] Optimized the elution gradient to allow the peak to elute in a clear region of the chromatogram and to shorten elution time to optimize peak shape.

[0023] Changed to a shorter HPLC column with smaller internal diameter and smaller particle size (ie switched from typical 150 mm x 4.6 mm, 5 micron packing to a 100 mm x 3.0 mm, 3 micron packing). This improvement in the method also improves peak shape and height and improves the method sensitivity. Columns of 50 mm x 2.1 mm, 3 micron packing may also be employed in the method for highest sensitivity requirements.

[0024] Technical details for the novel single seed analysis method are provided in the description below.

Reference solutions

[0025] Reference solutions are prepared using reference material of known purity. In general multiple weights of approximately 0.1 grams is transferred to a 100 ml volumetric flask or a 2 oz. bottle. The weight is recorded from an analytical balance to four decimal places. The volumetric flask is filled to volume with an appropriate solvent (selected based on the solubility of the reference material with the most common solvent being acetonitrile for HPLC analysis and methyl isobutyl ketone (MIBK)or acetone containing an internal standard such as dimethyl phthalate for GC analysis). The solution is sonicated or manually shaken until all the reference material is dissolved. This serves as a stock solution. An appropriate amount of this stock solution is serially diluted using a volumetric pipette and another volumetric flask. For analysis of multiple active ingredients a stock solution for each reference material is made, then all are combined by adding the appropriate amount by volumetric pipette into a fresh volumetric flask.

[0026] The second volumetric flask is filled to volume with an appropriate solvent (generally 50:50 acetonitrile water: 0.1% acetic acid for HPLC analysis and MIBK or acetone for GC analysis) This second dilution step should be identical in composition to the solvent used to extract the seeds as outlined below. Again, the solution is sonicated or manually shaken to ensure homogeneity. For consistency with the sample preparation below the solution is filtered using a disposable syringe and filter as its transferred into an autosampler vial. Typically this would be a 0.45 micron Acrodisc filter. The type of filter depends on the solvent used.

Single seed preparation

[0027] Sixty individual seeds are transferred into separate scintillation vials using care not to disturb the seed coating (in general forceps or a scoopula are used). The weight of each seed is recorded using an analytical balance. The weight is recorded to four decimal places. An extraction solution is added using a 5 ml Hamilton autodiluter. The desired amount of extraction solution is precisely added to each seed. To this end a volumetric pipette operated manually is not recommended. In addition the precision of the autodiluter should be tested prior to its use to ensure it performs adequately.

[0028] The composition of the extraction solution depends on the type of seed, the solubility of the active ingredients applied to the seed and the analytical method that will be used. In general a 50:50 acetonitrile: 0.1% acetic acid mixture is used for HPLC analysis and MIBK or acetone containing an internal standard such as dimethyl phthalate is used for GC of many common active ingredients off the majority of seeds. Exceptions are many, however, and include corn seeds in general and the active ingredient of captan in specific. The volume of extraction solution added is typically 1-4 ml depending on the size of the seed. The solution needs to cover the seed completely to ensure complete extraction, yet should be minimized to yield the most concentrated solution. The sample is next sonicated followed if needed by mechanical shaking for a time adequate to completely extract the active ingredients off the seed and into the extraction solution. This time must be determined experimentally for each combination of seed plus active ingredient by assaying the final solution and evaluating for full recovery of the theoretical amount of each active ingredient. Typically 30 minutes of sonication followed by 15

minutes of mechanical shaking is sufficient time. The solution is lastly filtered into an autosampler vial using a disposable syringe and a syringe filter (example a 0.45 micron Acrodisc filter) unless the total volume of solvent is 1 ml or less. In that case a Whatmann Uniprep vial with built-in filter is used for the autosampler vial to prevent solvent loss within the filter. The filters are chosen based on the solvent being used for extraction.

Instrumental Analysis

[0029] The solutions in the autosampler vials are injected into the instrument using a volume of typically 15 microliters for an HPLC run and 3 microliters for a GC run. An autosampler is used as manual injections are not precise enough for the analysis. Separation of the active ingredients is achieved via HPLC or GC. Typical HPLC columns used would include Nucleosil C18, Prism RP, Inertsil ODS-3, Lichrospher NH2, Discovery C18 and many others. The packing material is selected based on the physical properties of the active ingredients being evaluated. Typical GC columns used would include DB-1, DB-5, DB-1701 and many others. Again the column is selected based on the active ingredients being determined. Appropriate parameters for consideration in HPLC include the length, internal diameter and particles size of the columns. In general a 15 cm or less column is used preferably with 5 micron particles or less and an internal diameter of 4.6 mm or less. In the cases of small seeds or seeds treated with low amounts of active ingredient it is desireable to evaluate the column choice to ensure the smallest peak can be detected.

[0030] Similarly for GC it is recommended to use a capillary column and not a widebore column to ensure narrow peak shape of the smallest peaks. Detection of the active ingredients is generally done with UV for the HPLC analysis, selected a wavelength optimized for the active ingredients being determined based on their UV response. In general either 265 nm or 230 nm has been shown to be suitable for most active ingredients studied. Other detection methods such as thermal conductivity or evaporative light scattering can be used as well. In general the detection for the GC analysis has been by FID. Other detectors such as ECD or TCD can be used as well.

Quantitation

[0031] Quantitation of the amount of each active ingredient is done by measuring the amount of each ingredient seen by the detector, in general by measuring the area of the peak seen. This area is compared to that of the reference solution using in general external calibration for HPLC and internal calibration for GC. An average calibration factor should be calculated based on multiple injections of the reference solutions.

EXAMPLES

[0032] Commercial products for seed treatment were mixed with water and optionally a colorant at laboratory scale according to label instructions. Seed treatment was performed with a Hege 1l Seed Treater (Hege Equipment, Inc., 13915 W. 53rd Street N., Colwich KS). One kilogram of seed were added for each trial. The rotation speed of the Hege was set at 60 rpm. Once the correct rotational speed was achieved, the slurry was added through a syringe over 5 seconds, followed by a 30 second mixing period. Seeds were allowed to air dry for 24 hours prior to analysis.

Example 1- Analysis of imidicloprid, metalaxyl and captan from corn seeds.

[0033] A batch of corn seed was treated with imidicloprid, metalaxyl and captan (applied as Gaucho®, Allegiance® and Captan® 400, respectively) with a target concentration of 500 ppm imidicloprid, 20 ppm metalaxyl, and 460 ppm captan. The samples were analyzed as described below and the resultant seed-to-seed distribution curve for this treatment are shown in Tables 1, 2 and 3 for imidicloprid, metalaxyl and captan, respectively.

[0034] Eluent preparation (0.1% acetic acid in water): Add 1.8 ml of glacial acetic acid to 1800 ml of deionized water. Stir well, filter and degas prior to use.

Part A imidicloprid and metalaxyl standard and sample prepration

[0035] Stock standard preparation for imidicloprid and metalaxyl: Accurately weigh in duplicate (standard A and B) 0.0400- 0.0500 g of imidicloprid primary standard and 0.0300- 0.0400 g of

metalaxyl primary standard into separate 2-ounce bottles. Add 50 mL of a 50:50 mixture of acetonitile: deionized water. Sonicate for 30 minutes and mechanically shake for one hour.

[0036] Standard preparation for imidicloprid and metalaxyl: Add by pipette 5 mL of imidicloprid stock solution and 5 mL of Metalaxyl stock solution to a 250 mL volumetric flask. Add all A weights to one flask and label A, add all B weights to a separate flask and label B. Fill each flask to volume with 50:50 water: acetonitrile. Invert several times to mix.

[0037] Individual corn seed sample preparation for determination of imidicloprid and metalaxyl: Transfer one corn seed into a scintillation vial. Add 2.0 mL of deionized water. Allow to stand undisturbed for 30 minutes. Add 2.0 mL acetonitrile. Sonicate 30 minutes and mechanically shake for one hour. Filter with a 0.45 micron syringe filter prior to analysis.

Part B Captan standard and sample preparation

[0038] Stock standard preparation: Accurately weight in duplicate (standard A and B) 0.0900-0.1100 g of captan primary standard into a 2-ounce bottle. Add 50 ml acetone. Sonicate for 30 minutes and mechanically shake for one hour.

[0039] Standard preparation for captan: Add by pipette 5 ml of stock solution into a 250 ml volumetric flask. Fill volume with acetone. Invert several times to mix.

[0040] Individual corn seed sample preparation for determination of captan: Transfer one corn seed into a scintillation vial. Add 4.0 mL of acetone. Sonicate 30 minutes and mechanically shake for one hour. Filter with a 0.45 micron syringe filter prior to analysis.

[0041] INSTRUMENTATION

Perkin Elmer Series 200 LC pump or equivalent.

Perkin Elmer LC 235 Diode Array detector or equivalent.

Hewlett-Packard Series 1050 autosampler or equivalent

Hamilton MicroLab 1000 autodiluter or equivalent capable of delivering 25.00 ± 0.05 mL aliquots and 2.00 ± 0.05 mL aliquots.

LC Column – Prism RP 150 mm column with 4.6 mm internal diameter and 5 micron particle size.

Analytical balance with accuracy of ± 0.1 mg.

[0042] INSTRUMENT CONDITIONS

Detection:UV detection at 265 nm with 5 nm bandwidth and simultaneously UV detection at 230 nm with 5 nm bandwidth

Injection Volume: 10 µl

Flow: 1.0 ml/min

Column Temperature: 35°C

Run Time: Approximately 30 minutes

[0043] Gradient program (linear):

Time [minutes]	0.1% acetic acid [%]	acetonitrile [%]
0	85	15
5	85	15
15	25	75
18	25	75
21	85	15
25	85	15

[0044] Expected Retention Times:

Component	Retention time [Minutes]
Imidicloprid	10.4
Metalaxyl	17.1
Captan	19.5

[0045] TABLE 1 **IMIDICLOPRID RESULTS**

Percent of Average	Number of Seeds
0-4%	-
5-15%	-
16-25%	-
26-35%	-
36-45%	-

46-55%	-
56-65%	1
66-75%	7
76-85%	8
86-95%	11
96-105%	11
106-115%	10
116-125%	6
126-135%	2
136-145%	3
146-155%	1
156-165%	-
166-175%	-
176-185%	-
186--195%	-
196-205%	-
206-215%	-
216-225%	-
226-235%	-
236-245%	-
246-250%	-

(Average =421 ppm, Target = 500 ppm)

[0046] TABLE 2 **METALAXYL RESULTS**

Percent of Average	Number of Seeds
0-4%	-
5-15%	-
16-25%	2
26-35%	6
36-45%	7
46-55%	3
56-65%	3
66-75%	6
76-85%	1
86-95%	3
96-105%	4
106-115%	2
116-125%	4
126-135%	1
136-145%	3
146-155%	5
156-165%	1

166-175%	1
176-185%	3
186--195%	-
196-205%	1
206-215%	-
216-225%	1
226-235%	2
236-245%	1
246-250%	-

(Average = 21 ppm, Target = 20 ppm)

[0047] TABLE 3 **CAPTAN RESULTS**

Percent of Average	Number of Seeds
0-4%	-
5-15%	-
16-25%	-
26-35%	-
36-45%	-
46-55%	-
56-65%	2
66-75%	6
76-85%	16
86-95%	11
96-105%	5
106-115%	7
116-125%	4
126-135%	5
136-145%	-
146-155%	-
156-165%	3
166-175%	-
176-185%	-
186--195%	-
196-205%	-
206-215%	-
216-225%	-
226-235%	-
236-245%	-
246-250%	-

(Average = 626 ppm, Target = 460 ppm)

Example 2- Analysis of CGA-293343 (thiamethoxam), CGA-173506 (fludioxinil) and CGA-329351 (mefenoxam) and myclobutanil from cotton seeds.

[0048] A batch of cotton seed was treated with thiamethoxam (applied as Cruiser® 5FS), fludioxinil (applied as Maxim® 4FS), mefenoxam (applied as Apron XL® LS) and myclobutanil (applied as Systhane® WSP) with a target concentration of 3000 ppm, 25 ppm, 75 ppm, and 210 ppm respectively. TABLES 4-7 show the seed-to-seed distribution curve for this treatment.

[0049] Eluent preparation (0.1% acetic acid in water): Add 1.8 ml of glacial acetic acid to 1800 ml of deionized water. Stir well, filter and degas prior to use.

[0050] Stock standard preparation: Accurately weigh in duplicate (standard A and B) 0.0900-0.1100 g of CGA-293343 primary standard, 0.0450- 0.0550 g of CGA-173506 primary standard, 0.0450- 0.0550 g of CGA-329351 primary standard and 0.0450- 0.0550 g of myclobutanil primary standard into separate 2-ounce bottles. Add 50 mL 50:50 acetonitrile: deionized water. Sonicate for 30 minutes and mechanically shake for one hour.

[0051] Standard preparation: Add by pipette 40 mL of CGA-293343 stock solution, 3 mL of CGA-329351 stock solution, 1 mL of CGA-173506 stock solution and 8 ml myclobutanil stock solution to a 250 mL volumetric flask. Add all A weights to one flask and label A, add all B weights to a separate flask and label B. Fill each flask to volume with 50:50 water: acetonitrile. Invert several times to mix.

[0052] Individual cotton seed sample preparation: Transfer one cotton seed into a scintillation vial. Add 3.0 mL of 50:50 acetontrile: deionized water. Sonicate 30 minutes and mechanically shake for one hour. Filter with a 0.45 micron syringe filter prior to analysis.

[0053] INSTRUMENTATION

Perkin Elmer Series 410 LC pump or equivalent.

Perkin Elmer LC 235 Diode Array detector or equivalent.

Hewlett-Packard Series 1050 autosampler or equivalent

Hamilton MicroLab 1000 autodiluter or equivalent capable of delivering 25.00 ± 0.05 mL aliquots and 3.00 ± 0.05 mL aliquots.

LC Column – Prism RP 150 mm column with 4.6 mm internal diameter and 5 micron particle size.

Analytical balance with accuracy of ± 0.1 mg.

[0054] INSTRUMENT CONDITIONS

Detection:UV detection at 265 nm with 5 nm bandwidth and simultaneously UV detection at 230 nm with 5 nm bandwidth

Injection Volume: 10 µl

Flow: 1.0 ml/min

Column Temperature:35°C

Run Time: Approximately 30 minutes

[0055] Gradient program (linear):

Time [minutes]	0.1% acetic acid [%]	Acetonitrile [%]
0	85	15
5	85	15
20	25	75
23	25	75
26	85	15
30	85	15

[0056] Expected Retention Times:

Component	Retention time [Minutes]
CGA-293343	5.9
CGA-329351 (Mefenoxam)	16.6
Myclobutanil	19.4
CGA-173506	20.2

[0057] TABLE 4 **Thiamethoxam results**

Percent of Average	Number of Seeds
0-4%	-

5-15%	-
16-25%	-
26-35%	-
36-45%	-
46-55%	-
56-65%	1
66-75%	4
76-85%	18
86-95%	12
96-105%	4
106-115%	6
116-125%	5
126-135%	3
136-145%	4
146-155%	2
156-165%	-
166-175%	-
176-185%	-
186--195%	-
196-205%	1
206-215%	-
216-225%	-
226-235%	-
236-245%	-
246-250%	-

(Average = 2457 ppm, Target = 3000 ppm)

[0058] TABLE 5 **Mefenoxam results**

Percent of Average	Number of Seeds
0-4%	-
5-15%	-
16-25%	-
26-35%	-
36-45%	-
46-55%	2
56-65%	8
66-75%	10
76-85%	13
86-95%	7
96-105%	2
106-115%	3

116-125%	6
126-135%	3
136-145%	2
146-155%	1
156-165%	-
166-175%	-
176-185%	2
186--195%	-
196-205%	-
206-215%	-
216-225%	-
226-235%	-
236-245%	-
246-250%	-
>250%	1

(Average = 73 ppm, Target = 75 ppm)

[0059] TABLE 6 **Fludioxinil results**

Percent of Average	Number of Seeds
0-4%	-
5-15%	-
16-25%	-
26-35%	-
36-45%	-
46-55%	-
56-65%	1
66-75%	9
76-85%	12
86-95%	11
96-105%	7
106-115%	3
116-125%	7
126-135%	4
136-145%	4
146-155%	1
156-165%	-
166-175%	-
176-185%	-
186--195%	1
196-205%	-
206-215%	-

216-225%	-
226-235%	2
236-245%	-
246-250%	-

(Average = 21 ppm, Target = 25 ppm)

[0060] **TABLE 7** **Myclobutanil results**

Percent of Average	Number of Seeds
0-4%	-
5-15%	-
16-25%	-
26-35%	-
36-45%	-
46-55%	-
56-65%	1
66-75%	7
76-85%	15
86-95%	12
96-105%	4
106-115%	7
116-125%	2
126-135%	5
136-145%	4
146-155%	2
156-165%	-
166-175%	-
176-185%	1
186--195%	-
196-205%	-
206-215%	-
216-225%	-
226-235%	-
236-245%	-
246-250%	-

(Average = 196 ppm, Target = 200 ppm)

What is claimed is:

1. A method for determining the single seed loading distribution (Gaussian and non-Gaussian) of a pesticide (applicable to multiple pesticides) on a batch of pesticidally treated seeds (works with pigment-free, non-fluorescent compositions) comprising the steps of:

1) Selecting a subset of seeds from said batch sufficient to determing said distribution;
2) Maintaining a seed from said subset in contact with an extracting fluid in a sufficient volume to comprise a test sample and for a sufficient time to substantially non-selectively extract said pesticide from said seed;
3) Filtering the test sample containing the pesticide to substantially remove undesired substances extracted from the seed while maintaining a sufficient volume for HPLC;
4) Separating the pesticide from other substances in the filtered test sample by HPLC;
5) Illuminating the pesticide with UV light {other detectors e.g., FID (flame ionisation detector when GC (gas chromatography) is used.};
6) Detecting the UV absorbed by the pesticide;
7) Relating the amount of UV absorbed to a quantity of pesticide;
8) Repeating Steps 2-7 sequentially, or concurrently, for each seed in said subset;
9) Determining the single seed loading distribution (both Gaussian and non-Gaussian) for said batch based on the pesticide quantity determined for each seed in the subset.
10) Repeat Steps 4-9 to determine quantity and seed loading distribution of each additional pesticide in the test sample.

ABSTRACT

The present invention provides methods and devices for the direct quantitative analysis of multiple pesticides on individual seeds with or without fluorescent pigments. The seed varieties include corn, cotton, wheat, soybean and canola.